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CAMERA LUCIDA METHOD OF DIRECT HEMOCYTOMETRY WITH THE NEW HEMO-PROTRACTOR

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The earliest attempt to measure the diameter of blood cells was made by Thomas Young (1773-1829) who in 1813 successfully used the principle of diffraction, which is based upon the production of spectral coronas, the so-called "diffraction patterns," when a parallel beam of white light passes through a blood film. By means of an instrument which he called an eriometer, Young[1] estimated the average diameter of human erythrocytes to be 6.8 microns. But for years the diffractometric method attained no degree of popularity, for it was not until over a century later that Pijper[2] rediscovered the usefulness of the method in constructing his diffraction micrometer, or "blood cell tester" (Carl Zeiss). Since then several workers have utilized this principle in devising various types of instruments, such as Emmons' eriometer[3], Eve's halometer[4], Bock's erythrocy-

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tometer[5], Falsi's micro-diffractometer[6], and finally Haden-Hausser erythrocytometer.[7]

However, a serious objection to this method was advanced by Bergansius[8], who pointed out that diffraction of the cells as opaque bodies was not in reality the cause of the spectral rings, but that the latter resulted from interference by the images of the light sources produced by the erythrocytes acting as little lenses. On the other hand, Millar[9] was able to show, by presenting a limited series of data obtained by his specially devised apparatus, that this fundamental criticism set forth by Bergansius was not entirely correct. Nevertheless, after a careful discussion of the principle of diffraction in its mathematical details, Millar was led to conclude that the method could not be considered reliable for the determination of the absolute size of erythrocytes.

The only method which yields completely accurate and reliable results, in estimating the average diameter of blood cells, appears to be that of direct measurement of the cell size by the use of a micrometer under suitable and standardized conditions. Though first used by Ewell[10] in 1890, it remained for Price-Jones[11] to popularize this method of direct erythrocytometry. In the first account of his work Price-Jones used a camera obscura, projecting the cell image on to a table at a magnification of 1000 diameters. The outline of the cell to be measured was drawn in pencil and the size was measured with a millimeter scale. In his later publications, however, the cell image was projected upon a calibrated screen at an exact magnification of 2000 diameters, the measurement being made directly from the ruling drawn on the screen. The actual diameters of at least 500 red blood cells in microns were plotted and the resulting curve of distribution has come to be known as the Price-Jones curve.

However, Price-Jones' method as well as the values he obtained for average erythrocyte diameters were critically analyzed by Ponder and Millar[12] who, using a micro-photographic method, reported a difference of at least 1 micron between the cells suspended in plasma and those seen in dried smears. But to the contrary, Ohno and Grievius[13] found practically no difference in the average diame-

ter of red cells contained in plasma (7.98 microns) and of those on dried smears (7.99 microns).

Additional reports on the use of direct erythrocytometry by Hampson and Shackle[14], Grosh and Stifel[15] and McCormick[16] all seem to agree on the accuracy of the method. Slight alterations in the technic were introduced by Passey and Braine[17] who projected the cell image by means of a Zeiss photographic microscope; by Sauder and Toomey[18] who used Bausch & Lomb euscope; and Hynes and Martin[19] devised a celluloid protractor with calibrated circles for matching blood cells.

Although the technic of direct measurement of cell diameter is accurate, the chief objections raised against its use are: (1) it is time-consuming, (2) the projection method requires the use of a dark room, (3) the photographic method is tedious and expensive, and (4) the values are unreliable. For these reasons the method of direct erythrocytometry, like the diffraction method, has never become an established practice in average clinical laboratories today. The determination of packed cell volume by a suitable hematocrit appears to be the most generally employed means of measuring the dimensions of red blood cells. It must be remembered, however, that the measurement of cell volume is not identical with the estimation of cell diameter, for these are two different attributes of a blood cell. Undoubtedly in most instances the hematocrit method yields the essential data, but there are many other conditions in which the measurement of cell diameter is important for an accurate diagnosis and prognosis. Both determinations are requisite in a complete record of any disease in which anemia is a prominent and crucial symptom.

From the foregoing review of literature it appears evident that the simplest and yet the most reliable method is that of direct hemocytometry, as originally advocated by Price-Jones and using a camera lucida as a projector of cell image. His method of outlining each individual cell in pencil, however, is time-consuming and hence inadvisable. The use of a simple hemo-protractor, as described in the following paragraphs, has been found to be most convenient and much time may be saved by measuring only 100 well-preserved and

relatively spherical cells. When a little experience is gained in the use of this technic such measurements can easily be completed in the length of time usually required for making a differential leucocyte count.

Procedure

Method of Adjusting Camera Lucida to Hemo-Protractor. Since there exists an appreciable amount of difference in magnifying power of each objective, it is impracticable to devise a hemo-protractor which can be made universally applicable. For this reason one needs to construct a protractor according to the specific magnification scale obtained by the particular optical system employed in the microscope. The following procedure is recommended:

A camera lucida with an arm length of approximately 3 or 4 inches is first attached to the upper end of the monocular tube and the reflecting mirror is adjusted at an angle of 45° (Fig. 1). Then, by using an oil immersion lens, one of the small squares in the red cell counting area of a hemocytometer chamber is focused, a drop of clear immersion oil being applied on the ruled area of the counting chamber with no cover glass. When the square in question is in clear focus the four corners of the square are then accurately marked on a piece of white cardboard resting upon the table. The magnified square resulting from connecting these four points represents a particular magnification obtained by the optical system employed, each side of the square being a magnified line for 50 microns. The square so drawn is shown in Figure 2. The exact magnification may now be calculated by measuring the dimensions of the magnified square by means of a millimeter scale. Using the same scale of magnification and a fine point pen with India ink, the graduated scale of the protractor such as represented in the accompanying figure may be drawn. The hemo-protractor so prepared is used consistently in all subsequent measurements of cell diameter.

Method of Measuring Blood Cells. Once the hemo-protractor is accurately constructed the actual measurement of cell diameter is relatively a simple procedure. The cell image is projected on to the surface of the protractor, which is then freely moved on the table

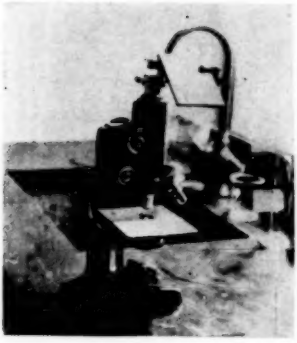

Fig. 1. Photograph of a microscope with camera lucida attached to its monocular tube.

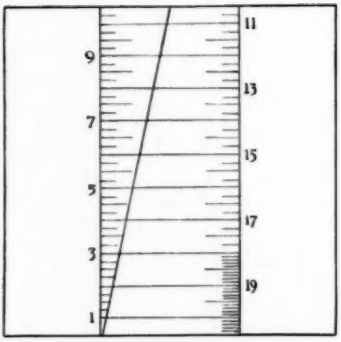

Fig. 2. Hemo-protractor constructed according to directions given in the text. The outer square represents a magnification, exactly 1000 diameters, of one of the small squares, actually 50 x 50 microns, in the red cell counting chamber. The protractor scale has been drawn accurately to the same scale of magnification with appropriate sub-divisions into halves and quarters, except in the right lower portion where the scale is subdivided into one micron. Each figure on both sides of the scale designates the distance between the outer vertical line and the central diagonal line, and indicates the diameter of the cell when it is accurately superimposed.

until the cell outline fits snugly between the two lines (one vertical and the other diagonal) of the scale and the diameter directly read off as marked on the margin (Fig. 2). For the average routine practice, and unless for any reason the estimation of the diameter of a greater number of cells is indicated, it is usually sufficient to measure 100 cells. All measurements of the blood cells may be expressed in increments of either 0.25 or 0.5 micron. A specially devised blank suitable for recording the results of measurement may be advisable for permanent files (Table 1).

Blood smears which are routinely made for differential leucocyte counts and stained by any Romanowski stain are entirely satisfactory for the purpose of measuring the erythrocyte diameter. It is to be emphasized, however, that the film should be carefully spread so as to avoid agglutination and rouleaux formation, which obscure the clear cell image. As a rule thin films of blood are preferable to thick preparations.

Some of the results obtained by the use of this method as above outlined are summarized in the accompanying tables (Tables 2 and 3).

Summary

A simple method of direct hemocytometry, with special reference to the measurement of average erythrocyte diameter, using a camera lucida and a specially devised hemo-protractor, is described. Since the instrument required is relatively inexpensive and embodies other features of usefulness, and the procedure extremely simple, the method is found to be adaptable for frequent and even routine usage in average clinical laboratories.

TABLE 1

Distribution Curve of Cell Diameters
(Erythroblastic Anemia)

Microns							Total	Per Cent
3.0	++++	'''					8	4.0
3.5	+++						3	1.5
4.0	++++	''					7	3.5
5.0	++++	++++	''				12	6.0
5.5	++++	'''					8	4.0
6.0	++++	++++	++++	''			17	8.5
6.5	++++	++++	++++	++++			20	10.0
7.0	++++	++++	++++	++++	++++	''''	29	14.5
7.5	++++	++++	++++	''''			19	9.5
8.0	++++	++++	++++	++++	++++	++++	30	15.0
8.5	++++	++++	++++	++++	''		22	11.0
9.0	++++	++++	''''				14	7.0
10.0	++++	++++	'				11	5.5
				Total			200	100.00

Mean cell diameter = 7.03 microns

TABLE 2

Diameters of Human Erythrocytes in Various Conditions

Conditions	Diameters Minimum	Maximum	Average	Number of Cells Counted
Normal adult	5.5	9.5	7.5	200
Normal infant	7.5	12.0	8.5	200
Macrocytic anemia (adult)	6.0	11.0	8.5	150
Microcytic anemia (adult)	4.5	7.5	6.0	200
Aplastic anemia (child)	6.0	8.0	7.0	100
Pernicious anemia (adult) (Liver therapy)	3.0	10.0	7.5	100
Congenital hemolytic anemia	4.0	9.0	7.2	100
Reticulocytes from above	5.0	8.5	7.0	100
Erythroblastic anemia (Macrocytic type)	5.5	11.0	9.0	200
Erythroblastic anemia (Microcytic type)	4.0	9.0	6.5	100
Erythroblasts from above	7.0	15.0	10.0	100
Ovalocytosis	3 x 6	7 x 11	5 x 9	50

TABLE 3

Red Cell Diameters in Normal Animals

Species	Diameters in Microns			Number of Cells Counted
	Minimum	Maximum	Average	
Goat	2.0	4.0	3.0	100
Sheep	3.5	6.0	4.7	100
Mouse	5.0	6.0	5.5	50
Guinea Pig	5.0	7.5	6.4	100
Pigeon	6 x 12	7 x 14	6.5 x 14	50
Frog	15 x 14	15 x 14	15 x 14	50

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NOTES ON TESTS OF URINE IN SULFANILIMIDE THERAPY, I

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The urine of individuals, subjected to sulfanilimide therapy, develops marked increases in acid concentration, with a corresponding increase in solids, as noted from higher specific gravities obtained.

Urine from each of six patients was collected, prior to the treatment with sulfanilimide, and these urines were tested for acidity, specific gravity, sulfanilic acid and for carbonates. In each case the urine was but slightly acid, the specific gravity was normal, and the tests for sulfanilic acid and carbonates were negative.

Urines from the same patients after they had received treatment with sulfanilimide, were also collected and subjected to similar tests as above. The quantity of sulfanilimide given was 40 grains per diem, and this without the use of bicarbonates, in order to note the conditions in the urine. The results obtained, showed that the acid concentration was great, the specific gravities were all higher, and each gave tests which were positive for sulfanilic acid and carbonates. The quantities of urine collected from these patients before and during the sulfanilimide treatment, were in general approximately the same.

The results found in the changes in the various specific gravities, are as follows:

	GROUP I Specific Gravity of Urine @ 60° F. before Sulfanimide Treatment	GROUP II Specific Gravity of Urine @ 60° F. after Sulmanilime Treatment
Urine from Patient No. 1	1.017	1.027
" " " " 2	1.015	1.023
" " " " 3	1.005	1.025
" " " " 4	1.010	1.018
" " " " 5	1.010	1.020
" " " " 6	1.016	1.021

When the administration of sulfanilimide was halted, the specific gravities of the patients were found to be lowered or normal, and were quite similar to those found in Group I.

Again, when the sulfanilimide treatment was resumed, the specific gravities were found to be like those under Group II.

Tests for the Presence of Sulfanilic Acid

10 cc of urine is placed in a test tube. To this is added 3 cc of 50 percent C. P. Acetic Acid, 2 cc Sodium Nitrite solution and then 2 cc of an acetic acid solution of alpha-Naphthylamine. The development of a reddish coloration is a positive indication of sulfanilic acid (and/or forms of sulfonic acid). Because of the sensitivity of this test, one (1) cc or less of the urine, is often a sufficient quantity.

In making the test for sulfanilic acid in urine, use is made of the procedure used in detecting and determining the presence and quantity of nitrous acid or nitrites in water, foodstuffs, and the like, by the development of the reddish coloration, being the formation of "azobenzolnaphthylamine sulfonic acid" produced whenever sulfonic acid and naphthylamine acetates (or chloride, etc.) are added to nitrous acid or an acidified solution of nitrites.

Preparation of Test Solutions

(1) a-Naphthylamine acetate solution. Dissolve 0.5 grams of the solid a-Naphthylamine in 100 cc of 5 normal Acetic Acid. Filter the solution through washed absorbent cotton.

(2) Sodium Nitrite solution. Dissolve 20 grams of Mercks (or similar) C. P. Stick Sodium Nitrite in 100 cc distilled water.

Test for Presence of Carbonates

Place 10 cc of urine in a test tube, and add 1 cc concentrated Hydrochloric Acid. An effervescence of gas, denotes the presence of carbon dioxide. Samples of urine from Group I gave negative tests, while samples of urine from Group II gave positive tests.

Presence of Excess Acids

Place 100 cc of urine in small beaker, and add 5 grams of C. P. Sodium Bicarbonate. The development of foaming together with

considerable gas formations, are indications of the presence of excessive or abnormal acid concentration. Samples of urine from Group I gave negative results with this combination of tests, while samples of urine from Group II gave positive tests.

All samples of urine from patients in Group II, were kept for two months. They were quite clear, of yellowish-brown color, and of course, quite acid.

When samples of urine from Group II were made slightly alkaline, to a pH of 8.0, the urines became cloudy and flakey substances were deposited. After standing for two weeks, long needle-like crystals were formed, varying from one-half inch to one and one-half inches in length.

Comment

The question arises as to the cause of acidosis during the course of the sulfanilimide treatment. Is it due to the interference of the buffer carbonates in the blood of the patient whose urine gave positive tests for carbonates in the presence of some organic acids? And, is the cyanosis due to carbon-dioxide, which may be liberated when it comes in contact with acids; or, is it due to carbon-dioxide and sulfanilimide together?

When more carbonates enter the system than can be neutralized, the blood appears to be more acid. When sulfanilimide is given, the acidosis is increased; more so, if the foods or drugs containing sulfur are given. In this connection, the influence of the presence of sulfur compounds is not known as yet.

The amount of sulfanilimide given to a patient is not enough to increase the specific gravity of the urine.

Further studies are now being carried out to determine the causes for the increased solid matter as well as the acid concentration in the urine during the sulfanilimide therapy.

THE DETECTION OF SUGAR IN URINE*

A brief historical sketch of the subject and a report of 10,000 comparative tests

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The history of the examination of the urine and the gradual evolution of the various chemical tests is very interesting. As the time is short I shall leave many interesting details for a longer report. Beginning with the classic period of Greek medicine we find Hippocrates (460-370 B.C.) teaching the importance of uroscopy which is the art of diagnosing disease by the inspection and examination of the urine. He observed the quantity, general appearance, consistency and color and amount of sediment. If the urine was black the patient was considered in a dangerous condition. If the patient showed signs such as frothing at the mouth, convulsions and dim vision he was considered in a critical condition and death inevitable.

Galen (131-201 A.D.) was interested in the diseases of the urinary tract. He recognized diabetes only by polyuria and thought it was due to a loosening of the kidney structure.

In the middle ages urine was thought to be a distillate of the blood formed in the liver and combined with other juices derived from the metabolic processes. The science of uroscopy or water casting became very popular. Many quacks flourished. They travelled the country on a pony and led the people to believe that they could diagnose every ill and fortell all kinds of events, by examining the urine. The urine was examined in a special type of glass, of a characteristic shape, similar to the bladder, so that the urine would be in its natural position. This bottle was carried in a suitable covered basket. Often the glass was graduated at various levels. The level of the sediment or of the putrefactive cloud was

* *Read before the American Society of Medical Technologists, New York City, June, 1940.*

supposed to indicate different ailments in the body. The urine glass became the symbol of the Medical art. The painters and wood engravers devoted much of their time to portraying the physician, in a very solemn manner, inspecting the urine while the well dressed patients looked on. The details of the Dutch painters are especially interesting where beautiful costumes and accessories depict as much of the life of the times as the physician who holds the urine glass up to the light.

Lorenzo Bellini (1662) discovered that if he evaporated the urine he changed the color, taste and odor. He realized that these properties depended upon the quantity of water present.

Thomas Willis (1674) was among the first to place importance on the chemical contents of the urine. He noted that diabetic urine had a characteristic sweet taste.

Shakespeare refers to the practice of uroscopy along with his other allusions to the medical practices of his day.

Matthew Dobson (1776) discovered that diabetic urines contained sugar and that the blood serum of diabetics had a sweet taste. A little later Cowley isolated sugar in a free state and started the quantitative examination of the urine.

William Cruickshank (1798) noted that the sugar in the urine of diabetic patients shows a different reaction from milk sugar.

Michel-Eugéne Chevreul (1815) observed that the sugar of diabetic urine resembled grape sugar and was not like cane sugar.

Prout (1820) made many interesting discoveries about the composition of urine. He also found that the sugar in the urine of diabetics was not common sugar, but similar to that found in grapes.

In 1836 Rees published one of the first text books on the scientific analysis of urine. He measured the amount of sugar by evaporation and incineration.

In 1847 the first handbook for urine analysis was published by Markwich. He mentioned the detection of sugar by fermentation and the copper test which Trommer had published in 1841. Trom-

mer added copper to the urine, then an excess of alkali, and boiled the solution, noting the color changes. He distinguished between the cane and grape sugars. The test was not very satisfactory as it was not stable.

Hermann von Fehling in 1848 improved this test for sugar in urine. He stabilized the amount of copper and alkali so that the solution could be used for the quantitative determination of sugar in urine. Fehling's solution is used today as a qualitative test for sugar.

W. S. Haines of Chicago (1874) modified Fehling's test so that the cupric hydroxide was held in solution by glycerol instead of Rochelle salt.

E. Niylander (Nylander) published a paper on an alkaline solution of Bismuth as a reagent for grape sugar in urine, in 1882, in Scandinavia. It was reprinted in 1883 in German. He made a solution of Bismuth subnitrate, Rochelle salts and Potassium hydroxide. Five cc. of urine and ½ cc. of reagent were heated 5 minutes in a water bath and cooled. Sugar reduced the solution to black by the precipitation of the bismuth. Before proceeding with the determination of sugar, albumen had to be removed by heating and filtering. Large amounts of uric acid and creatinine interfered with the test.

Many other tests have been developed to determine the presence of sugar in urine, using copper sulphate solutions. The most popular today is the one which was published in 1911 by S. R. Benedict. Various other chemicals have been utilized to show the presence of sugar. One of these is the method introduced by J. B. Sumner in 1925. Dinitrosalicylic acid is used in the reagent. The urine is heated with the solution and the resulting yellow color compared with standards.

The returns of the A. S. C. L. T. questionnaire of 1935, sent out to hospitals throughout the country, shows the following methods used for the qualitative determination of sugar in urine:

Benedict's	68%	Sumner	3%
Haines	7%	Nylander	3%
Fehling's	5%	Miscellaneous	14%

Technique and Discussion of Galatest

Galatest is a fine, white powder, put up in small narrow glass vials by the Denver Chemical Company. It was developed by Alexander Galat, in their laboratories. It is a modification of the Nylander test. It contains a bismuth salt and caustic soda and is very hygroscopic If the cap of the vial is screwed on tightly, the powder appears to keep indefinitely.

The test is very simple to perform. A small amount of the powder is shaken out on a piece of paper, in a cone shaped pile. A very small drop of the urine to be tested is placed in the middle of the cone from a finely pointed glass dropper. The reaction is complete in less than 30 seconds. If sugar is present, the powder will turn light grey, dark grey or black depending upon the amount of sugar present. After making a number of determinations, one can tell quite accurately whether this amount of sugar present is less than 0.2% (powder is pale grey) or about 0.5% (powder is dark grey) or 1% or over, (powder is black).

This observation is based upon 10,000 comparative tests performed during the winter of 1939. Benedict's qualitative test was used as a control. The results of the Galatest checked with the Benedict's test. Maternity specimens giving a positive reaction were checked by the fermentation method to differentiate between glucose and lactose. The Galatest powder did not show a positive reaction unless there was about 2% lactose present. The fact that Galatest does not give false positive reactions in many discolored urines is a great advantage.

In our laboratory all urines showing the presence of sugar are tested by a quantitative method to determine the exact amount present. If one wishes to guess at the amount, one may do so as accurately with this Galatest powder as with Benedict's solution. As our routine analysis set up calls for 8 specimens to a set, I place six rows of eight piles of the powder on a piece of half size paper (8½ x 5½) and the tests are completed in 5 minutes. The color or type of paper does not matter, but very thin paper will curl if 48 tests are done on it.

This Galatest method is very much quicker and cheaper than any other. Routinely we get about 200 tests from a vial of powder. Purchased by the dozen vials, each vial costs about thirty-five cents. This makes each test cost about one and a half mills.

Benedict's is the most popular method for the routine examination of sugar in the urine. It is the cheapest and fastest of the copper sulphate methods. Even when the chemicals are purchased in quantity, the cost per pint is approximately 40 cents. The standard test uses 5 cc. of the reagent which makes the test cost four mills. There is also the cost of test tubes used. A certain amount of time is required to place the 5 cc. of reagent in the test tube. This probably takes about as long as it does to shake out the Galatest on the paper. The eight drops of urine must be measured out. The one drop may be placed on the Galatest powder in less time. The tubes with the Benedict's solution must be placed in the water bath, boiled 5 minutes and removed. The tubes must be emptied, washed and either set up for another set of tests or dried and put away for future use. Also, a certain amount of time and equipment is required to make up the Benedict's solution.

The following chemicals added to routine urines did not give a false positive test with Galatest powder:

Ammonia	Phenol
Chloroform	Phosphoric acid
Cholesterol	Merthiolate
Cresol	Oxalic acid
Hydrochloric acid	Sulphuric acid
Nitric acid	Thymol

Xylol

The only chemical added which gave a false positive test was formalin. This is due to the fact that it is an aldehyde and therefore a reducing agent.

The following substances present in urines in pathological conditions did not give a false positive reaction:

Acetone	Methylene blue
Albumen	Silver nitrate
Bile salts	Cystine
Blood	Leucine
Creatinine	Tyrosine
Diacetic acid	Various crystals
Indican	Sulfamids as:
Urobilin	prontysil
Urobilinogen	sulfanilamide
Urea	sulfapyridine
Uric acid	sulfathiazol
Acriflavin	sulfamethylthiazol

Summary

1. A short history of the evolution of the tests for sugar in urine is given.
2. A report is given of 10,000 routine urines examined for sugar by Benedict's method and Galatest powder. The results checked perfectly.
3. Large amounts of various chemicals found in pathological urines, or added to urines did not give false positive reactions with Galatest.
4. Galatest is very much faster and cheaper to use than any other known method.

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THE SEPARATION OF BLOOD SUB GROUPS A_1, A_2, AND A_1B, A_2B*

By BEATRICE TOHARSKY

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The purpose of this paper is to present the method for the separation of blood sub groups A_1, A_2, A_1B, and A_2B as it was developed by Davidsohn.[1] That differentiation which until now was made by means of elaborate and complicated methods, is of importance for medico-legal purposes and as Davidsohn and others believe as a means of avoiding blood transfusion reactions or of explaining reactions, whose cause may be obscure.

Ten years after Landsteiner ascertained the existence of human blood groups, von Dungern and Hirschfeld[2] reported that the A or II group was not homogeneous. They had adsorbed group O and group B serums with certain of the group A cells. Their adsorbed serums still clumped the majority of group A cells even while it did not clump cells like those used in the adsorption process. The blood groups A and AB were thus split into the subgroups A_1, A_2, A_1B, and A_2B. The cells of the subgroups A_1 and A_1B clump readily and strongly with anti-A serums while the cells of subgroups A_2 and A_2B clump weakly. If the anti-A serum is not of sufficiently high titer they may not be clumped at all and the A_2 cells may be mistaken for O cells, and the A_2B cells may be mistaken for B cells. The proportion of A_1 bloods to A_2 bloods is about four to one. The proportion of A_1B bloods to A_2B bloods is somewhat less. Until recently it was assumed that the A_1 and the A_2 bloods were entirely compatible. There have, however, been numerous cases reported to show that this assumption was ill founded. For details of these

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* *Read at the Seventh Annual Meeting of the Illinois Society of Clinical Laboratory Technicians at Champaign-Urbana, November 11, 1939.*

cases I can refer you to Davidsohn's paper in the *Jornal of the American Medical Association.*[1]

The nature of the difference between the subgroups is a disputed one. It is believed by such workers as Landsteiner and Levine[3] that the difference is a qualitative one. This view is supported by the existence of agglutinins for A_1 cells in A_2 and A_2B serums and of agglutinins for A_2 cells in A_1 and A_1B serums. The anti-A_2 agglutinin is rare and possesses the property of clumping not only A_2 cells but also O cells. Often it clumps the latter more strongly than the A_2 cells. Recently we received a blood of group B in our laboratory, which possessed an agglutinin for group O cells. By means of adsorption tests, it was shown that this was an anti-A_2 agglutinin. The importance of this is obvious. Sometimes a universal donor is used without a cross agglutination of patient and donor bloods. In this case the patient's serum would have clumped the donor's cells with what may have been dire results.

Investigators such as Lattes[4] base their belief that the difference is quantitative upon the fact that the agglutinins may be removed from anti-A serums by the adsorption of these serums with either A_1 or A_2 cells. When the latter are used as the adsorbing agent, they must be used in much larger volume. Too, more than one adsorption may be necessary to accomplish the removal of all the agglutinins.

Perhaps the greatest significance of these findings lies in the danger of not recognizing an A_2 blood and of calling it an O blood. This is especially likely to occur when a weakly agglutinating grouping serum is used. This mistaken impression might be substantiated by the action of the serum, for the serum of such an A_2 blood may simulate an O serum, since it may contain in addition to the normally present anti-B agglutinins, anti-A_1 agglutinins. If such a blood is given to an individual of group B or O, both of which have anti-A agglutinins, a severe reaction is likely to occur. If the recipient's isoagglutinin titer is low, even a cross matching of donor and recipient bloods may not detect the error, for the same weaknesses would be inherent here as were present in the original grouping. The danger is especially imminent in cases of leukemia, frequent

cells of the subgroup A_1 will remove completely the anti-A agglutinins. The quantity may be as small as 1/32 of the volume of the serum dilution. Large volumes of the cells of the subgroup A_2 (even one half of the volume of the serum dilution) will adsorb the agglutinins only partially. According to Friedenreich and Worsaae[8], there are no transitions between the subgroups. However, this method is cumbersome and time consuming, requiring at least three hours. It, too, requires a great deal of that hard to get high titer group B serum. A large quantity of packed cells is also required and these are not easily available. As a routine test before transfusions, it is not very practical.

To be of value as a routine procedure for blood grouping, the method for the differentiation of the blood subgroups must be reliable but at the same time rapid and relatively simple. It occurred to Davidsohn to attempt the solving of the problem by the utilization of the common antigenic fraction of the human blood of group A and of sheep blood. The serum of Schiff and Adelsberger, although it identified the group A cells was not specific enough to differentiate the subgroups. Davidsohn noticed that Schiff and Adelsberger were able to remove the anti-A agglutinins from their serum by adsorption with boiled sheep cells, and he decided to inject rabbits with boiled sheep cells in the expectation that the serums produced would act differently toward the cells of the different subgroups. The serums which resulted did react with the different subgroups in specific dilutions. By the utilization of this fact the test was devised to differentiate the subgroups. In the preparation of the serums, rabbits were given four to five injections of a 20 per cent suspension of sheep cells which had been boiled one half hour. Control animals were injected with raw sheep cells. Of the rabbits injected with the boiled sheep cells, about 45 per cent produced subgroup specific serums. The animals injected with raw cells also produced anti-A agglutinins. However, the subgroup specificity was not present in the latter serums, that is, the serum clumped the A_2 cells in a dilution as high as that which clumped A_1 cells. Thus the animals injected produced three kinds of serum, that which was specific enough to be used for the differentiation of the subgroups, that which had an anti-A titer so low that it could not be used, and that which although

recipient's, for these have been shown by Davidsohn[5] to have low isoagglutinin titers.

Thus, two problems arise for the individual who is grouping bloods for transfusion work: the assurance of the recognition of the A and AB groups when the cells are of the A_2 and A_2B subgroups, and the separation of the subgroups.

The answer to the first problem lies in the use of high titer anti-A serums. These serums are often not available. Davidsohn[5] found that the isoagglutinin titer rises in individuals who have received an injection of horse serum and he recommended for typing the use of serums from patient's with serum disease. Another source of high titer anti-A serums is from rabbits which have been immunized with sheep cells. Schiff and Adelsberger[6] found that the group A cells and sheep cells had a common antigenic factor. With this principle in mind they injected rabbits with sheep cells and obtained a serum which agglutinated red cells of group A in much higher dilutions than the cells of groups O and B. Such a serum has two advantages over the use of the human isoserum. It has a high titer and it is readily produced.

Several methods have been recommended for the solution of the second problem, the differentiation of the subgroups. Most of these methods are impracticable and all are tedious to perform. With the method of von Dungern and Hirschfeld[2], group B serum is adsorbed with A_2 cells. The serum then clumps only A_1 cells. For this method high titer serums of group B are necessary. There the difficulty lies in obtaining such serums. Large volumes of A_2 cells must be available and these too are difficult to obtain.

Landsteiner[7] and his associates recommended the use of the irregular agglutinin anti-A_1 for the recognition of the subgroup A_1 and of the agglutinin anti-A_2 for the recognition of the subgroup A_2. These agglutinins are relatively rare and are usually too weak to be reliable.

The procedure which is recognized at present as the standard method is the one in which the serum of group B is adsorbed with varying quantities of red cells of the group A. Small amounts of

it produced a high titer anti-A serum could not be used to differentiate the subgroups.

With the specific serums available, a technic was sought which would (1) permit prompt recognition of the feebly agglutinating subgroups A_2 and A_2B as well as of the strongly agglutinating subgroups A_1 and A_1B but would not agglutinate either the group B or the group O cells; (2) would differentiate the subgroups clearly and unmistakeably; (3) would be simple and easily carried out; (4) and would be rapid enough to be applicable as a routine procedure under emergency conditions such as exist before a blood transfusion. The slide test was devised to answer all these needs.

The rabbit serums which acted specifically with the subgroups were titrated that their so-called group titers and subgroup titers might be determined. The group titer is the dilution of the serum which will clump all of the group A and the group AB cells while it will not clump B or O cells. The subgroup titer is the dilution of the serum which will clump A_1 and A_1B cells while it will not clump A_2 and A_2B cells. The serums were inactivated at 55° C. for thirty minutes, and the dilutions of the serums were made in test tubes. Approximately two per cent suspensions were made of A_1, A_2, B, and O cells by the addition of one drop of the whole blood to one c.c. of physiologic saline. Two drops of the serum dilution were placed on a slide within a circle made with the colored wax pencil. A drop of the cell suspension was added. The slides were tilted back and forth a few times and then were placed under petri dishes under which bits of moist cotton were also placed in order to avoid excessive evaporation. The slides were then examined macroscopically and microscopically at the end of five, ten, fifteen, and thirty minutes. The dilutions representing the desired titers, that is the group and subgroup titers were then selected. With the group titer there was distinct, macroscopically visible clumping with A_1 and A_2 cells at the end of five minutes, while there was detected no clumping even microscopically of group B and group O cells. With the subgroup titer, the A_1 cells were clumped at the end of five minutes, the clumping being visible to the naked eye, while even at the end of thirty minutes the A_2 cells were not clumped or were

clumped very weakly, the clumping being visible only microscopically.

After the titers of the serums have been fixed, the technic of determining the subgroups is relatively simple to perform. It consists merely of taking a glass slide, marking two circles on it with the wax pencil, and adding to one circle two drops of the serum which has been diluted to its group titer and to the other circle two drops of the serum in its subgroup dilution. A drop of two per cent suspension of cells is added to each serum dilution. The slides are tilted back and forth as in the titration technic and the results are read in five minutes. If there is distinct clumping on both sides of the slide, the cells are those of subgroup A_1 or A_1B. If there is clumping of the cells with the serum diluted to the group titer and if there is an absence of clumping with the subgroup dilution of the serum, the cells are those of subgroups A_2 or A_2B. If clumping does not occur on either side, the cells may be said to be either of the groups B or O.

When the subgroups of A and AB were determined by this method, other methods were also utilized for the purpose of comparison. The cells were typed with human serums. This was the accepted method of Vincent. It soon became apparent that this technic was not dependable. The difference in agglutinability with the human serums did not differentiate the subgroups, for sometimes A_2 and A_2B cells were clumped more strongly than the cells of subgroups A_1 and A_1B. The adsorption method was also employed. B anti-A serums were adsorbed with two volumes of A cells. Cells of subgroup A_1 removed all the anti-agglutinins even when a volume as small as 1/32 of the volume of the serum dilution was used. Cells as small as ⅓ of the volume of the serum dilution was used. Cells of subgroup A_2 failed to do this even in one half volume. This is the most reliable confirmation of the blood subgroup. The subgroups of the bloods as determined by the rabbit serum dilution method in every case concurred with the results of the adsorption test.

Summary

Available serologic data suggest that subgroups A_1 and A_2, A_1B and A_2B are not always compatible; and that subgroups A_2

and A_2B are not infrequently mistaken for bloods of other groups, particularly for O and B.

Davidsohn's method for the recognition of the subgroups has been described. This offers two advantages. The high titered, easily produced, and highly specific rabbit immune serum permits prompt recognition of the blood groups A and AB, including the feebly agglutinating subgroups A_2 and A_2B. A proper dilution of the serum as determined by titration makes it possible to differentiate the subgroup A_2 from A_1 and A_2B from A_1B. Both procedures can be carried out within five minutes.

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THE NEUFELD TEST FOR PNEUMOCOCCUS TYPING: SOME OF THE DIFFICULTIES ENCOUNTERED IN ITS USE, WITH MICROSCOPIC DEMONSTRATIONS*

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For six or eight years now the Neufeld test has served as a principal cornerstone in the fight against pneumonia. In fact, prompt, dependable type diagnosis has become such an essential feature of pneumonia programs that it is doubtful whether any considerable success could have been attained without the use of this test.

The spectacular advent of sulfapyridine and its related antecedent compounds has raised a question in the minds of many as to whether serum therapy will give way to chemotherapy and the Neufeld reaction, therefore, lose its importance. Our experience with sulfapyridine has been too short as yet to define its limitations. The increasing use of serum as a supplement to sulfapyridine, suggests that combined sero- and chemotherapy may become the method of choice. Whatever the future of the Neufeld test may prove to be, there has been no reduction in the demand for typing service in our laboratories and I imagine the same is true in other laboratories represented here.

The principles of the test may be stated very briefly. Thirty types of pneumococci have been recognized and are designated by numbers from 1 to 32, excluding 26 and 30. Each type is serologically distinct allowing for some cross-reactions, and type specific sera have been prepared against each. Mixtures of these specific sera have been made in order to reduce the number of slide preparations required in typing. Six mixtures are used most commonly, designated by letters A to F, inclusive.

Typing consists in examining microscopically the mixtures of

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sputum first with each of these serum pools and then with the individual sera comprising any pool which yields a positive reaction. A dye, usually methylene blue, is added to each preparation to facilitate reading the result.

A positive Neufeld reaction consists chiefly in (1) the pronounced swelling of the transparent capsule surrounding the pneumococcus and (2) precipitation of the polysaccharide of which the capsule is composed, rendering the latter opaque or translucent. The capsule typically takes on a glistening appearance which makes it stand out brightly in its field. It remains unstained with the exception of its rim, whereas the cell body stains intensely under optimum conditions.

In negative preparations (mixtures of pneumococci with heterologous sera) capsules may be of fair size and have stained rims, but the distinctive swollen, glistening opaque appearance is absent.

We have recently become interested in extending the Neufeld technic to include the diagnosis of pneumobacillary infections.

Several different types of Friedlander bacilli, as you are no doubt aware, have been recognized. Julianelle who has done the most extensive taxonomic work in this group of organisms found three types which he calls Types A, B, & C. The types discovered by earlier workers have not, to the best of my knowledge, been brought into correlation with these three. Still other types possibly remain unidentified as is certainly the case with the pneumococci.

The Friedlander bacilli appear not to be so frequent producers of pneumonia as was thought twenty or twenty-five years ago. Just how important they are is not an easy question to answer, since so simple a test as the Neufeld method has not been available. Possibly some instances of Friedlander pneumonia have been overlooked or laid at the door of non-typable pneumococci. With this in mind and because of the occasional occurrence of extrapulmonary Friedlander infections we have prepared type-specific sera for applying the Neufeld technic to diagnosis of Julianelle's three types of pneumobacilli. We have learned recently that the New York City Diagnostic Laboratory has used this method for typing pneumobacilli for the past two years, but have not published their experience with it. It is my understanding also that serums will soon

be commercially available for diagnosing the Julianelle types by the Neufeld procedure.

The technic does not differ at all from the usual Neufeld test except that since less satisfactory titers are obtainable against Friedlander antigens the use of ample proportions of serum is the more important. This applies more especially to the pool of the three sera than to the individual sera themselves. We have not been able so far to produce sera answering to the titer requirements established by the National Institute of Health for diagnostic anti-pneumococcus sera. This is in keeping with the experience of Julianelle (personal communication) who found Friedlander bacilli poor antigens in the production of type specific agglutinating sera. We have adopted tentatively the following standards:

(1) Type-specific sera must produce maximum capsular swelling up to a dilution of 1/5 or higher. Most of them do so in 1/10 dilution or higher.

(2) The pools of Types A, B, and C sera must produce maximum capsular swelling in each of the types up to a dilution of 1/3 or higher.

Our sera have not been in use long enough to justify much discussion of their practical value. So far four positive typings have been made. Three of these have proven to be Type A infections and one a Type B. One of the Type A specimens was a prostatic exudate. The other three were from pneumonias. Type A infections are generally considered to be most common and most severe.

The morphology of Friedlander bacilli is quite variable under conditions of culture. While quite long bacillary forms are not uncommon and capsules are typically large, cultures are sometimes seen in which the capsules are small and the diplo-bacillary form so abbreviated that confusion with pneumococci would be perfectly easy without knowledge of the identity of the serum used.

We have not had a large enough series of specimens as yet to enable us to say whether pneumobacilli are equally variable under natural conditions. If such is the case, it probably means that some pneumonias have been reported as caused by non-typable pneumococci which were of pneumobacillary origin. A Gram's stain would, of course, serve to correct such an error.

The difficulties most commonly encountered in the performance of Neufeld tests are those involved in failures to type. It is true that cross-reactions are a possible source of erroneous positive reports, but regulations of the National Institute of Health require testing for cross-reactions and removal of cross-reacting antibodies by absorption.

Variations in capsular size are also a source of confusion particularly in laboratories where the number of specimens examined is so small that adequate acquaintance with all of the types is slowly acquired. Errors from this cause may be on either the positive or negative side.

Some cross-reactions are more common than others. The outstanding example is that of Type III and VIII. The capsular polysaccharides which determine type specificity are quite closely related in these two types and a sufficiently prolonged period of immunization against Type III antigen may usually be depended upon to produce a relatively high agglutinin titer and capsular swelling against Type VIII pneumococci. The converse also holds for Type III cross-reacting titers when Type VIII immunizations are prolonged.

Reciprocal cross-reacting tendencies are also said to occur in less degree in the case of Types I and VII and in Types II and V. other cross-reactions, usually non-reciprocal, occur occasionally and unpredictably. They may in most cases be avoided if the immunizing process is so carried out as to arrive quickly at the peak of the antibody curve. In spite of this, however, and even the fact that National Institute of Health regulations require the removal of cross-reacting antibodies, occasional experiences in typing lead one to suspect that some cross-reacting serums escape detection. For example last spring in our laboratory two mixed infections were encountered within a month of each other involving Types XXIX, XX and a third type which was different in the two instances. The duplication of Types XXIX and XX was of such interest to us that the second sputum was examined with typing sera produced by a different manufacturer. In this instance Type XXIX sera produced swelling, but Type XX serum did not. A second set of typing sera, preferably from a different source, is a useful precaution

when the number of typings is sufficient to justify it.

Opposite extremes in capsular size are usually seen in Type III which is notoriously large and Type V which is typically small. Other types than III occasionally present equally large capsules. So long as the observer is guided by the glistening, turgid, opaque appearance and not by size alone, there is little danger of reporting erroneous positives in these large-capsuled varieties. The smallness of the Type V capsule, however, does present a real problem, since even when swollen it is often not so large as in negative preparations of some other types. I know of some technicians who will not report a sputum as containing non-typable pneumococci without first testing against Type V serum even though all the serum mixtures have given negative results.

We have in one specimen encountered a large-capsuled Type V pneumococcus. The Cooper strain of Type III has a small capsule. Inconstancy of capsular size among the several types can be discovered in any laboratory where pneumonia sputums are frequently examined.

The most disconcerting cause of failure to type is found in cases treated with sulfanilamide and sulfapyridine. There is some disagreement regarding this fact, but the bulk of the experience reported, I believe, corroborates the observation that specimens fail to type when the patient has been under treatment long enough with sulfanilamide or one of its derivatives. How long the drug may be administered before producing this effect I am not able to say. It is probably related to the circulating level more dependably than to the interval after the beginning of administration. Mouse inoculation is effective in some cases at least in restoring the capsules and their capacity to react typically. It is sometimes necessary, however, to pass the organisms through two or three mice before typical morphology is restored.

One difficulty is experienced by the State laboratories which is of less importance in local laboratories. I refer to the problem of typing relatively old specimens. We receive a rather large (too large) percentage of sputa through the mail. Sometimes even though the specimen is brought in by a messenger the date of collection shown on the specimen card reveals that it has stood around somewhere for a day or two before being sent to the laboratory.

Both pneumococci and pneumo-bacilli undergo autolysis with relative speed. Some types autolyze more quickly than others. If a sputum contains a Type I pneumococcus, for example, it can usually be typed after two or three days or even longer exposure to room temperature. If it contains Type III pneumococci, however, the latter will usually have disintegrated so completely after overnight exposure to room temperature that typing will be difficult or impossible.

The great importance of fresh specimens in the satisfactory typing of pneumonia is one of the main reasons for which the Illinois Department of Public Health has employed every means to encourage the use of local typing laboratories by physicians. There are other advantages, of course, which accrue when local typing facilities are available, such as the ease of obtaining repeated specimens which in the case of blood cultures may serve as a prognostic guide and as an indicator of the adequacy of treatment.

One source of error in typing may arise from the very zeal with which a technician endeavors to find capsular swelling in as many sputa as possible, in other words, to have as few untyped specimens as possible. The normal presence of typable pneumococci in many throats should be kept in mind, and good judgment exercised in reporting when only a few scattering organisms have shown swollen capsules. Otherwise serum may be administered for a pneumococcus which is not a factor in the patient's infection, involving unnecessary cost and confusing the statistics of efficacy of serum therapy.

We have not exhausted the list of difficulties by any means. However, with the exception of the interference caused by sulfapyridine and related compounds none of them obscures results in a serious proportion of cases. By and large the Neufeld reaction is a very dependable one and reduction in a small number of erroneous or questionable results is as much as can be expected from a discussion of this sort. If this is accomplished I shall be more than pleased.

Demonstration—capsular swelling of a Type C Friedlander bacillus by its homologous serum.

EDITORIAL

AN APPEAL TO MEDICAL TECHNOLOGISTS

Are you enjoying fully the privilege of being an Medical Technologist?

Are you availing yourself of the opportunities which are offered our professional organization (The A. S. M. T.) by the A. S. C. P., and the medical profession in general?

Are you joining other medical technologists who are working individually and collectively as the A. S. M. T., toward a fuller realization of our aims and purposes?

If not, just consider for a moment the unlimited opportunities for service and personal development that you are ignoring.

We did not choose our chosen field of work because it seemed the easiest thing to do or because it offered fame and a great fortune. We chose to be medical technologists because of an irresistible force that seemed to hold us when we first saw a malarial parasite or a minute organism that had produced death and even plagues, or saw for ourselves the reactions in our test tubes that would eventually influence the chance for life or death of a fellowman. Perhaps you do not feel particularly fired with the glory of your every day work, but only such a spirit will hold a technologist to his work day after day and make him a credit to his profession.

It is so easy to fall into a rut, unless we keep up to date, and inspired through contacts with the outstanding leaders in medical science. Think what inspiration it would give you to meet the men who have developed laboratory medicine, who have worked out the technics we use, and have written the texts which we study and use for references daily. You can have that inspiration. Through the efforts of the A. S. M. T. these men are made available to us at the annual conventions. We have the privilege of their counsel and

advice in the solution of our personal problems. These conventions are always held in medical centers, concurrently with the A. M. A. meeting, so that we have the opportunity to observe other laboratories, and pick up valuable hints on technic and apparatus. Through the courtesy of the A. M. A. we have the privilege of visiting the A. M. A. exhibits, which are a revelation to medical science.

The A. S. M. T. Journal is filled with a wealth of material applicable to our every day problems. It is such a simple means of keeping up to date. Moreover you can contribute the ideas which you have found valuable in carrying out your own work.

Registration is a privilege bestowed upon us by the A. S. C. P., and membership in the A. S. M. T. is a responsibility and an opportunity to show our appreciation for our profession through cooperative effort with other medical technologists toward the realization of the goal we have set for ourselves. Start doing your part today, not by just wishing that you could do something to help your profession, but by membership and taking an active part in its program.

BOOK REVIEWS

GOULD'S POCKET PRONOUNCING MEDICAL DICTIONARY. By the late George M. Gould, A.M., M.D., revised by C. V. Brownlow. Eleventh edition, 1939. P. Blakiston's Son & Co., Inc., 1012 Walnut Street, Philadelphia, Pa. Flexible, washable fabric, $2.00; with thumb index, $2.50.

Over 40,000 principal words used in medicine and the collateral sciences are pronounced and defined. In addition there is an appendix containing complete tables of the arteries, bones, chemical elements, hormones, micro-organisms, muscles, nerves, prefixes and suffixes, phobia's signs and symbols, etc. A physicians' dose-list with their incompatabilities and a veterinary dose-table are included both in English and metric measures. These are based upon the eleventh revision of the U. S. Pharmacopoeia. Many new words are found in this edition. The inclusion of the prefixes and suffixes greatly enhances the utility of the dictionary as many words can be modified and new meanings evolved. It is claimed by one of the authors that in this little dictionary many sound medical terms are included which cannot be found in any other medical dictionary.

GRADUATE MEDICAL EDUCATION. Report of the Commission on Graduate Medical Education, 1940. Pp. 303. Composed and printed by the University of Chicago Press, Chicago, Ill.

This most interesting report prepared by the Commission on Graduate Medical Education after almost three years of study will be welcomed by undergraduates and practitioners alike. It serves as a companion book to the Final Report of the Commission on Medical Education published in 1932 which, however, dealt largely with problems of undergraduate medical education. In this report graduate medical education is discussed from the standpoint of the internship, the residency, the specialty boards and postgraduate

medical education for specialists and for general practitioners with a final chapter on studies made in these fields in Great Britain.

The senior medical student about to enter an internship all too frequently must rely on meager information relative to an internship in general. The report of the Commission is the answer to many questions in the mind of the undergraduate who doesn't know where to turn to find accurate information. Practitioners will find the chapter on Postgraduate Medical Education most illuminating. Specialists desirous of certification by the Board in their particular specialty will be interested in the brief history of the Specialty Boards as well as information relative to the preparation necessary to obtain certification. Informative tables and some existing plans of postgraduate education are given in the appendix.

In this review no better summary of the objects of the Commission can be given than in the last paragraph of the foreword. "The ultimate objective of this report is to stimulate greater interest in the whole field of graduate medical education so that there will be improved educational content of the internship and residency resulting in a better trained medical profession with a keener desire to keep abreast of developments in medical practice and with a clearer realization that medical education is a continuous process extending throughout the physician's lifetime. The essential purpose of all these recommendations is to provide better medical care to the patient."

NEWS AND ANNOUNCEMENTS

The Board of Registry has just conducted the biggest semi-annual examination on record. 621 applicants were admitted to the examination in October, 1940, under 149 clinical pathologists in various cities throughout the United States, Hawaii, Canal Zone, and Canada. In addition, there were many applicants who had to be denied examination at this time due to lack of fundamental qualifications.

The questions used in the written examination were as follows:

WRITTEN QUESTIONS FOR EXAMINATION OCTOBER, 1940

(Maximum Time for Written Examination—3 hours)

I. Assuming normal blood has 5,000,000 red cells per cmm., 15.6 gms. of hemoglobin per 100 cc. representing 100% hemoglobin, a color index of 1, a packed cell volume of 48% of the total volume, and a volume index of 1 then:
   A. How many *grams of hemoglobin* is represented by 80% hemoglobin?
   B. What % *hemoglobin* is represented by 11.7 gms. of hemoglobin?
   C. If the red cell count is 4,000,000 with 80% hemoglobin, what is the *color index?*
   D. If the red cell count is 2,500,000 with a packed cell volume of 29% of the total volume, what is the *volume index?*

II. A. Define:
   1. Anisocytosis
   2. Anisochromia
   3. Megaloblast
   4. Metamyelocyte
   5. Antigen

B. What is the average diameter of an erythrocyte?

III. A. What instructions and advice do you give patients in order to have them in basal condition for a metabolism determination?

B. Outline the steps in preparing a piece of fresh tissue for microscopic diagnosis by *one* of the following methods:

1. Frozen section
2. Paraffin embedding

IV. A. What are the steps in staining a fresh smear for bacteria by Gram's method?

B. What are the constituents of:

1. Gram's iodine solution
2. Carbolfuchsin
3. Wright's stain

V. A. Two drops of 0.5% alcoholic solution of dimethylaminoazobenzol added to 5 cc. of gastric fluid produced a yellow color. Three drops of 1% alcoholic solution of phenolphthalein were added and the mixture titrated to neutral by the addition of 1 cc. of decinormal (N/10) solution of NaOH.

1. What is the Free Hydrochloric acid?
2. What is the Total Acicity?

B. How may pepsin or pepsinogen be detected in gastric fluid?

VI. A. A specimen of sputum contains small greyish-yellow granules detected by the unaided eye. What important finding may be revealed by microscopic examination of these granules?

B. Outline the technic for examining a specimen of sputum for tubercle bacilli by the Ziehl-Neelson staining method.

VII. A. How many cc. of tenth normal (decinormal) potassium hydroxide solution are required to neutralize 10 cc. of normal oxalic acid solution?

B. What temperature (state whether degrees Centigrade or Fahrenheit) maintained for how many minutes is advised for inactivation of complement in serum?

C. The atomic weights of the elements are:
Cl - 35; H - 1; K - 39; Na - 23; O - 16; S - 32.
What is the molecular weight of:

1. Sulphuric acid
2. Sodium chloride
3. Potassium hydroxide
4. Hydrochloric acid

VIII. A. For chemical examination of blood how do you obtain

1. Clear serum?
2. Plasma?
3. Noncoagulated whole blood?

B. Give the normal values of the following in blood?

1. Calcium (serum) in 100 cc.
2. Total nonprotein nitrogen in 100 cc.
3. Sugar in 100 cc.
4. Plasma protein

IX. A. Tabulate the structures sought for and the material to be examined in attempting to identify the following parasitic infestations:

	Structures Sought	Material Examined
(Example) Trichuris trichiura	ova	feces
1. Ascaris lumbricoides	.....	.....
2. Oxyuris vermicularis	.....	.....
3. Endamoeba histolytica	.....	.....
4. Malaria	.....	.....
5. Trichinella spiralis	.....	.....

B. How can Taenia saginata be differentiated from Taenia solium?

X. A. What laboratory procedures are available for the diagnosis of typhoid fever and when is each most effective?

B. List at least four laboratory procedures of value in the diagnosis of syphilis.

C. What laboratory procedures are of aid in diagnosing infectious mononucleosis?

XI. A. 1. What protein aside from albumin and globulin occurs in the urine in certain pathological conditions?

2. How do you test for this protein?

B. How do you test for acetone in the urine?
C. What is the total protein content of normal cerebrospinal fluid in mg. %?

The members of the American Society of Medical Technologists will no doubt join the Registry in their appreciation of the time, work and expense given by the Clinical Pathologists who have conducted the tests.

At the last meeting of the Board of Registry in June, 1940, a resolution was adopted that an Advisory Board be inaugurated whose membership was to be made up of two registered Medical Technologists who were also members of the American Society of Medical Technologists, and three members from the certificate holders at large. We are therefore pleased to announce that the following have been appointed and have signified their acceptance:

Miss Elizabeth Boksenbom, M.T., 10520 Pasadena Avenue, Cleveland, Ohio.
Mr. Allen Gold, M.T., 312 East 11th Street, Topeka, Kansas.
Miss Henrietta M. Lyle, M.T. (member American Society Medical Technologists), St. Joseph's Hospital, Lancaster, Pennsylvania.
Miss Jane McCurdy, M.T., 111 East 76th Street, Lennox Hill Hospital, New York City, N. Y.
Mr. Lawrence O. Ray, M.T. (member American Society of Medical Technologists, 108 East Central Avenue, Orlando, Florida.

LOOKING FORWARD TO THE 1941 CONVENTION

It is the wish of the Program Committee to help make the Cleveland Convention outstanding through its scientific papers. This can be accomplished only through the sincere efforts of each member. If you have a scientific paper to present or know of a member who needs a bit of urging, will you kindly get in touch with anyone of the Committee members?

The papers must be purely scientific with no clinical material included. Twenty minutes is the time allotted to each speaker. If

lantern slides or motion pictures are included, please specify the type. The names and addresses of possible guest speakers will also be appreciated.

For further information please write to any of the following members of the Program Committee or with the Chairman direct.

Committee—Frieda Claussen, 469 Laurel Ave., St. Paul Minn.; Rachel Lehman, Suite 912 Hulman Bldg., Evansville, Ind.; Evelyn Jardine, Mary Hitchcock Memorial Hospital, Hanover, N. H.; Dorothea Zoll, 1420 W. Girard Ave., Philadelphia, Pa., Chairman.

RESOLUTION AS ADOPTED BY THE BOARD OF DIRECTORS OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Whereas, the American Red Cross has undertaken the enrollment of Medical Technologists willing to serve in the Medical Departments of the United States Army and Navy, if and when their services may be required, during a national emergency, if and when such a condition exists, and

Whereas, the requirements for such enrollment are similar to those now required for registration by the Registry of Medical Technologists of the American Society of Clinical Pathologists, not taking into consideration the gradual increase in the requirements for eligibility for registration with said body and the ability, experience and value to their profession of those technologists who have qualified for registration before the requirements for said registration have reached the present high level, and

Whereas, the women meeting the requirements for enrollment are given civilian status,

Be It Resolved, that the A. S. M. T. views with deep concern this policy which discriminates against so many of its members and fellow medical technologists which will create a situation in which many of the most valuable medical technologists will not be able to offer his or her services or else will be forced to accept a position of inferior capacity, and

Be It Resolved, that the A. S. M. T. expresses its desire to have all medical technologists holding certificates of registration of the

Board of Registry of Medical Technologists of the A. S. C. P., as well as those having the proper requirements to register with that body (the latter being the requirements for enrollment with the Red Cross at this time) be acceptable for enrollment in the Army and Navy Medical Departments through the Red Cross, and

Be It Resolved, that the A. S. M. T. express its desire to have both men and women Medical Technologists enjoy the privileges and compensations in keeping with their professional status by having military ratings, and

Be It Further Resolved, that this resolution offered by the Board of Directors of the A. S. M. T. for that society, to be ratified by that society at its next meeting, be entered in the books of said society and copies sent to the Chairman of the Senate Military Affairs Committee, Chairman of the House of Representatives Military Affairs Committee, Chairmen of the Naval Affairs Committees of both the House of Representatives and Senate, Surgeon General of the United States Army, American Red Cross, Secretary of the American Medical Association, Secretary of the A. S. C. P., Members of the Board of Registry of Medical Technologists of the A. S. C. P., to all affiliated societies of the A. S. M. T. and to as many other state societies as possible.

SUMMARY OF REPORT OF THE COMMISSION ON GRADUATE MEDICAL EDUCATION

Important developments in the hospital internship, the hospital residency and the postgraduate educational opportunities for physicians in practice were suggested by the Commission on Graduate Medical Education, whose final report was published on June 25. The Commission, which was organized by the Advisory Board for Medical Specialties on December 4, 1937, is now bringing to a close its three-year study program. Its work has been financed by national foundations and interested professional organizations.

The internship, suggests the Commission, should be considered as a basic preparation for the practice of medicine. It should round out and give practical application to the medical school course and, hence, should be closely allied to undergraduate medical education.

It should prepare young physicians adequately to begin general family practice and should provide them with the essential preparation necessary to undertake further study leading to the practice of a specialty. It should not attempt to train men for the specialties directly and, therefore, the intern should not be given training in the detailed technics of the specialties.

To prepare the intern for general practice, he should have experience in internal medicine, pediatrics, obstetrics and gynecology, and surgical diagnosis, minor surgery and treatment of emergencies. Special attention in these fields should be given to preventive medicine and the care of chronic diseases, conditions of the aged and functional disturbances. The whole atmosphere should be educational in character and the intern should learn by example as well as precept.

The residency is defined by the Commission as a prolonged period of study in one of the special fields which can be properly classed as graduate education, whether an advanced degree is granted or not. The Commission warmly supports the recommendation of the specialty boards that adequate attention be given during the residency to the basic sciences as they relate to the various specialties. It suggests practical ways by which hospitals may provide this basic science training in their own laboratories or through arrangements with medical schools. The report suggests that there is danger that too many residencies may be developed and stresses that, in the best interests of the patient, high quality of teaching in the residency is now more important than a large increase in the number of residencies. The essentials of a satisfactory residency are listed in some detail, although the Commission takes pains to point out that it does not wish to standardize residencies or put them in a strait-jacket.

Postgraduate education the Commission defines as study intended to keep a physician abreast of his chosen field of practice but not intended to equip him to enter a new field. Separate and clearly defined types of work are recommended for general practitioners and for specialists. While there has been a marked and rapid increase in interest in the field of postgraduate medical education, there is still need for its further extension and for improvement in the type of opportunities offered. The report points out the advan-

tages and disadvantages of the various types of training now provided.

The effect of the work of the specialty boards upon the practice of medicine is discussed in the report, which points out that these boards have provided a well defined yardstick for measuring an individual physician's competence in his specialty. Men in the specialties have been certified so rapidly that it soon will be possible for the great majority of the people of this country to have access to the services of certified specialists.

The entire report stresses the value of adequate training and points out that this will be reflected in improved care of patients.

Arkansas

The Arkansas Society of Medical Technologists held its annual convention September 21, 1940, at the University of Arkansas School of Medicine.

A very interesting program was presented with Dr. L. W. Diggs of Memphis as the outstanding speaker. His subject was "Technical Advances in Hematology."

The following officers were elected for the coming year:

President—Marjorie Wade, Hot Springs; Vice-President—Lucille Scott, Little Rock; Secretary-Treasurer—Virginia Mims, Little Rock; Executive Committee—Violetta Wakefield, Fort Smith; Louise Vance, Pine Bluff; Ann Snow, Little Rock.

North Carolina

The North Carolina Society of Medical Technologists is two years old. The third annual meeting was held in Greensboro, North Carolina, April 27, 1940. At the business meeting in the afternoon routine and special business was attended to.

The committee named in 1939, Mary E. Matthews and Mary R. Hunter, to draw up a Constitution and By-laws for the Society presented their report. The Constitution and By-laws were accepted as drawn up by the committee.

Mary R. Hunter introduced Miss Annette M. Callan, formerly of Philadelphia now of Southern Pines, North Carolina. She spoke

informally to the meeting about the advantages as individuals and as a Society in belonging to the American Society of Medical Technologists. She urged that some sort of exhibit be sent from the North Carolina Society for the National meeting in June. (The North Carolina Society of Medical Technologists did send to the National meeting a booklet showing the Society's advances in its two years of existence.)

The following new officers for the coming year were elected: President, Mary R. Hunter of Raleigh; Vice-President, Edna Woody of Wilmington; Secretary, Sara Hodges of Valdese; Treasurer, Elizabeth Forbes Gorman of Winterville.

The business meeting adjourned until 6:30 at which time an informal banquet was held in the Green Room of the O. Henry Hotel. Guests at the banquet were Miss Lila Bell Love of the faculty of the Woman's College of the University of North Carolina, and Dr. N. F. Conant of Duke University Hospital. After the banquet Dr. Conant spoke on "Pathogenic Fungi."

NOTICE TO MALE ENROLLEES OF DRAFT AGE

The enrollment plan being conducted by the Red Cross at the request of the Surgeon General of the Army is to provide a list of eligible technologists for service in the Medical Departments of the Army and Navy in the event of a war in which the United States is involved or a threatened war which necessitates the declaration of a national emergency by the President.

Many male technologists enrolled with the Red Cross for this service are within draft age and accordingly registered on October 16th. The Office of the Surgeon General of the Army has advised that they have requested the military authorities concerned with the induction of trainees into the Army to classify for duty in the *Medical Department*, all technologists enrolled by the Red Cross for war service who are drafted for one year of compulsory military training. The Surgeon General's Office has also advised that *insofar as vacancies are available*, enrollees who are conscripted will be given the non-commissioned status provided for their technical spe-

cialty under the war service enrollment plan.

Local draft boards are responsible for determining the availability of men for military service by classifying them in groups in the order in which they are subject to call. They do not have any jurisdiction over the particular branch of the military service in which the men may be detailed to serve. This latter is determined by the military authorities after a man has been drafted. Accordingly, therefore, all male Red Cross enrollees who are drafted for service should display their enrollment card to the military authorities at the time they are actually inducted into the service and they should be assigned to duty in the Medical Department rather than in one of the other branches of the military service. It is also advisable to indicate the fact that they are enrolled with the Red Cross on the questionnaires sent them by their draft boards or if enrolled subsequent to returning the questionnaire, to advise their local board of their enrollment.

The above supersedes all previous information issued concerning medical technologists who are enrolled with the Red Cross for service in the event of war who may be inducted for training under the Selective Service and Training Act.

AMERICAN RED CROSS
National Headquarters,
Washington, D. C.

INDEX TO VOLUME VI

ABSTRACTS, 36, 83, 123,

ALMADEN, P. J., Ph.D., 108.

BACTERIAL dissociation. Some practical aspects on, P. J. Almaden, Ph.D., 108.

BAKER, MARIAN A., M.T., 235.

Basophilic Aggregations, Robert A. Glenn, M.D., 1.

BECTON, CLARA M., M.S., M.T., 60.

Blood Cell, Red, counts photoelectric-haemacytometer with correction factors for abnormal cells, Cecelia M. Kortuem, R.N., M.T., 199.

Blood sub groups A_1, A_2, and A_1B, A_2B, The separation of, Beatrice Toharsky, *269*.

Blood typing tool, An improved, Arthur T. Brice, B.A., M.T., 173.

Book Reviews, 39, 84, 128, 284.

BRICE, ARTHUR T., B.A., M.T., 173.

CONVENTION City, New York's World's Fair for 1940 adds interest to, 96.

DARKFIELD Examination, A new technic for, Theodore Sizemore, M.T., 120.

DODSON, ROGER V., M.T., 58.

EDITORIAL, 282.

FLINN, JOHN S., B.S., 11.

FORSTER, GEORGE, M.D., *276*.

GLENN, ROBERT A., M.D., 1.

GROSKIN, LORAINE, B.S., 150.

HADLEY, HENRY G., M.D., 35, 81, 112, 178.

Hematology, The usefulness and responsibilities of Medical Technologists in the practice of routine, Clara M. Becton, M.S., M.T., 60.

Hemocytometry with the new Hemo-Protractor, Camera Lucida Method of, Katsuji Kato and Cecelia M. Kortuem, R.N., M.T., 250.

Hookworm — History, Identification, and Modes of Infection, with Laboratory Reports in Fifteen Cases, Marian A. Baker, M.T., 235.

ICTERUS, Estimation of Mild, Henry G. Hadley, M.D., 112.

IKEDA, KANO, M.D., *222*.

JONIK, SISTER MARY GERARDA, 20.

KATO, KATSUJI, 250.

KEIPER, THEODORE W., M.A., M.T., 11, 180.

KORTUEM, CECELIA M., R.N., M.T., 199, 250.

LABORATORY, Charts and files in the pathological, Phyllis Stanley, M.A., M.T., 72.

Leucocyte Count, Factors Supporting the Filament - Non-Filament Differential, Ida L. Reilly, M.T., 113.

Liver Function Test, Choosing the, Frederick C. Narr, M.D., 51.

LOPEZ, AURELIO, M.T., 121.

MAGLIARO, LUKE, M.D., 260.

Method, Camera Lucida, of Direct Hemocytometry with the New

Hemo-Protractor, Katsuji Kato and Cecelia M. Kortuem, R.N., M.T., 250.

Methods of Counting Bacteria and an Extensive Bibliography on the Subject of Bacterial Counting, Sister Mary Gerarda Jonik, 20.

Method, An Hypobromite, Improved by Means of a New Ureometer for the Determination of the Urea-Quantity in Blood and Urine, J. Th. Peters, M.D., 160.

Methods for Securing Clear Sera, Henry G. Hadley, M.D., 35.

Method, A Rapid and Accurate, for the Determination of Urea in Blood, Roger V. Dodson, M.T., 58.

Method, The "Whipping", its Comparison with other Methods in Examining for Mycobacterium Tuberculosis, Theodore W. Keiper, M.A., M.T., 180.

Museum in a Small Hospital, The, Phyllis Stanley, M.A., M.T., 15.

NARR, Frederick C., M.D., 51.

New York, The Convention and Visitor Capital of the World, 145.

News and Announcements, 43, 85, 131, 189, 246, 286.

Notice, Special, to Medical Technologists, 94.

PAYNE, Violet, M.T., 69

Peters, J. Th., M.D., 160.

Photography, The Value of, in Technical Work, Violet Payne, M.T., 69.

Program, 134.

REILLY, Ida L., M.T., 113.

Registry, Twelve Years of, and its Contribution to Medical Technology, Kano Ikeda, M.D., 222.

Report of the Committee on Education and Research of the American Society of Medical Technologists, 184.

SERUM For Streptococcus Alpha Prime, Production of Immune, Dorothea Zoll, M.T., 102.

Sizemore, Theodore, M.T., 120.

Stanley, Phyllis, M.A., M.T., 15, 72, 263.

Sugar in Urine, Detection of, Phyllis Stanley, M.A., M.T., 263.

Sulfanilimide Therapy, Notes on the Tests of Urine in, I, Luke Magliaro, M.D., 260.

Syphillis in Blood, A Technique for a Rapid Report of, Aurelio A. Lopez, M.T., 121.

TEST, Choosing the Liver Function, Frederick C. Narr, M.D., 51.

Tests, Complement Fixation and Flocculation, With the Same Antigens, Henry G. Hadley, M.D., 178.

Test, The Neufeld, for Pneumococcus Typing: Some of the Difficulties Encountered in its Use, With Microscopic Demonstrations, George Forster, M.D., 276.

Test, Rytz, Henry G. Hadley, M.D., 81.

Tests of Urine in Sulfanilimide Therapy, Notes on the, I, Luke Magliaro, M.D., 260.

Toharsky, Beatrice, 269.

UREA in Blood, A Rapid and Accurate Method for the Determination of, Roger V. Dodson, M.T., 58.

Urea-Quantity in Blood and Urine, An Hypobromite Method, Improved by Means of a New Ureometer for the Determination of the, J. Th. Peters, M.D., 160.

VIRUSES, The Ultramicroscopic, Loraine Groskin, B.S., 150.

WATER, The Effect of Time and Temperature of Storage on the Results Obtained in the Bacteriological Analysis of, Theodore W. Keiper, M.A., M.T., and John S. Flinn, B.S., 11.

ZOLL, Dorothea, M.T., 102.

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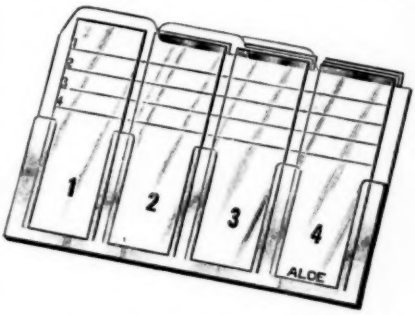

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